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A new record of *Ascidiella scabra* (Müller, 1776) (Ascidiacea, Phlebobranchia) in the southwestern Atlantic

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Abstract

Non-indigenous ascidians are transported across oceans in vessel-hull fouling communities, and regional traffic plays a role in their secondary spread. We found the ascidian *Ascidiella scabra* (Müller, 1776) in the hull-fouling community of an oceanographic vessel confined to waters of the southwestern Atlantic and Southern Oceans. The previously known distribution of this species was restricted to its native area (Mediterranean Sea and northeastern Atlantic); its presence in the southwestern Atlantic may have been masked in the past by the occurrence of its congener *Ascidiella aspersa* (Müller, 1776).

Keywords

Ascidians, biofouling, non-indigenous, regional traffic, secondary spread, shipping

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Introduction

Ascidians (Tunicata, Ascidiacea) are ubiquitous organisms in all marine environments (Shenkar and Swalla 2011). Although their short-lived larval stage limits natural dispersion (Stolfi and Brown 2015), the transport of adult specimens as encrusting fauna of vessels' hulls can enhance long-distance colonization by these organisms (Pérez-Portela et al. 2013; Castro et al. 2020; Meloni et al. 2020). The genus *Ascidiella* Roule, 1884 includes three species that are distributed throughout the Atlantic Ocean, i.e., *A. aspersa* (Müller, 1776), *A. scabra* (Müller, 1776), and *A. senegalensis* Michaelsen, 1914 (Rocha et al. 2012). Among these species, *A. aspersa*, which is native

to the North Sea and the northeastern Atlantic, currently has a wide distribution across the northwestern Atlantic and in Japan, Australia, New Zealand, India, and the southwestern Atlantic (Dybern 1969a; Tatián et al 2010; Nishikawa et al. 2019; Ma et al. 2019). This species can compete with native species for space and may affect their recruitment success (Osman and Whitlach 2000). *Ascidiella senegalensis* has a distribution restricted to its native area (northwest Africa), with few overall reports (Millar 1965). Finally, *A. scabra* is present in the Mediterranean Sea, the North Sea, and the northeastern Atlantic Ocean, where the species is considered native (Berrill 1950; Dybern, 1969b; Millar 1970; Ramos-Esplá et al. 1991; Nishikawa and Otani 2004).

High external morphological similarity has been reported between *A. aspersa* and *A. scabra* (Berrill 1950), eventually resulting in their misidentification. The distinguishing characteristics of the two species include the number of oral tentacles and longitudinal vessels of the branchial sac. While *A. aspersa* has fewer oral tentacles than longitudinal vessels, this relationship is inverted in the case of *A. scabra* (Lindsay and Thompson 1930). The number of oral tentacles is unrelated to the individual body length in either species (Nishikawa et al. 2014). Additional distinctive features include the number and size of outer follicle cells and the buoyancy of oocytes. In *A. aspersa,* follicle cells are larger but scarcer (28–32) than in *A. scabra* (70–80) (Lindsay and Thompson 1930). Additionally, while oocytes of *A. aspersa* float in seawater, those of *A. scabra* sink (Berrill 1928, 1950; Millar 1966, 1970; Nishikawa and Otani 2004). Nishikawa et al. (2014) considered the numbers of oral tentacles and longitudinal vessels key distinctions between both species, as the analysis of oocytes is impractical in immature, non-reproductive, or poorly preserved individuals. These authors also estimated that genetic differences were sufficient to support the two morphologically based entities. Unfortunately, reliable differentiation between *A. aspersa* and *A. scabra* require molecular analyses or detailed examination of internal anatomical characters involving the dissection of specimens have hampered or even led to erroneous species-level identifications in the past.

In the southwestern (SW) Atlantic, Monniot (1970) reported the presence of three poorly preserved specimens of *Ascidiella* sp. collected during a R/V *Calypso* campaign (1961–1962). More recently, the presence of *A. aspersa* has been reported in ports of the Argentine Patagonia, from San Antonio Este south to Puerto Deseado, although the species has not been found beyond there where lower water temperatures prevail (Tatián et al. 2010; Schwindt et al. 2014). The species was identified among undetermined museum collection specimens collected by Olivier and Bastida (unpublished data) in Punta Pardelas on the Patagonian coast in 1962 (Tatián et al. 2010). Colonization by non-indigenous ascidians other than *A. aspersa* has been detected in this large marine area (Orensanz et al. 2002). Recently detected species include *Diplosoma listerianum* (Milne Edwards, 1841) (Schwindt et al. 2014) and *Styela clava* Herdman, 1881 (Pereyra et al. 2015). Here we expand on the presence of *A. scabra* in the SW Atlantic, which was recently reported by Schwindt et al. (2020) based on a provisional presentation of the present results.

In a recent study, Meloni et al. (2020) described the invertebrate macro-fouling diversity present on the hull of an oceanographic vessel, R/V *ARA Puerto Deseado*, while anchored in the Port of Mar del Plata, Argentina. The sailing routes of this vessel covered a broad area extending 20 degrees of latitude along the SW Atlantic and the Southern (Antarctic) Ocean (Meloni et al. 2020). Hull-fouling communities on this ship are likely an integration of benthic fauna from many ports. This is also a strong potential vector for the regional dispersal of these species (Meloni et al. 2020). In that study, the presence of nine ascidian species was reported, and some specimens belonging to the genus *Ascidiella* were detected. However, species-level identifications could not be accomplished (Meloni et al. 2020). Our purpose is to conduct a detailed morphological analysis of these specimens collected to obtain species-level identifications, which might reveal its introduction in a little studied region of the world's oceans.

Methods

We re-examined specimens attributed to the genus *Ascidiella* which were collected from underwater surfaces of R/V *ARA Puerto Deseado* and analyzed in Meloni et al. 2020). Specimens were collected from the leading edge of the rudder (5 individuals), dry dock support strip (patches on the hull's bottom where dry-dock blocks supporting the vessel while out of the water were located, and which were therefore devoid of antifouling coatings), bow thruster, and sea chest gratings while the vessel was in the water, and the bulbous bow during dry-dock sampling (a single individual in each location) (see Meloni et al. 2020 for sampling details; collection permit: Servicio de Hidrografía Naval, SIHN, ODE N°19/20). Specimens were fixed in ethanol and deposited in the collection of the Museo de Zoología, Universidad Nacional de Córdoba (MZUC).

We dissected, analyzed, and photographed specimens using a microscope camera (OMAX A35140U) attached to a stereomicroscope (Labomed CZM4 and CZM6). We inspected general features, paying special attention to those described by Nishikawa et al. (2014), including the number of longitudinal vessels in the branchial sac and the number of oral tentacles. We stained specimens with Bengal Rose for detailed observation. We compared our observations with the available descriptions of *Ascidiella* spp. (Berrill 1950; Monniot and Monniot 1972; Nishikawa and Otani 2004; Nishikawa et al. 2014). We then obtained known previous records of the species from the Global Biodiversity Information Facility (GBIF 2020) and created a global distribution map using the

QGIS software (QGIS.org 2021, using the World Mercator EPSG: 54004 projection).

Results

Family Ascidiidae Herdman, 1882
Genus *Ascidiella* Roule, 1884

***Ascidiella scabra* (Müller, 1776)**
Figures 1A, B, 2A–C

Distribution. NE Atlantic, South Atlantic (Schwindt et al., 2020), Mediterranean Sea and North Sea (Berrill, 1950; Millar 1970; Nishikawa and Otani 2004; Rocha et al. 2012; Nishikawa et al. 2014) (Fig. 1A).

New records. ARGENTINA • Buenos Aires Province, Navy Base of Mar del Plata; 38.0348°S, 057.5352°W; 9 Sept. 2011; N. Correa and F. Sylvester leg.; in-water samplings performed by scuba divers (see Meloni et al. 2020 for sampling details); MZUCVI 00001, 1 spec. • Mar del Plata SPI Shipyard dry-dock; 38.0504°S, 057.5354°W; 14 Sept. 2011; N. Correa and F. Sylvester leg.; specimens collected in different hull locations, when ship was in dry dock (see Meloni et al. 2020 for sampling details); MZUCVI 00002 to MZUCVI 00009, 8 spec.

Identification. Solitary, oval ascidians measuring 2–5 cm in total length. Tunic strong and translucent; in some cases with small, pointed papillae projecting from tunic and randomly distributed over body. Branchial and atrial apertures with 8 and 6 lobes, respectively. Zooids with strong musculature (Fig. 2A), much concentrated in siphons, with both circular and longitudinal muscle fibers. Oral tentacles 17–36, simple, filiform (Fig. 2B). Dorsal tubercle U-shaped, with both horns more or less curled inwards (Fig. 2B). Dorsal lamina ribbed, with an even margin (Fig. 2, B). Branchial longitudinal vessels in the pharynx variable in number. Right side with 15–30 longitudinal vessels; left side with 12–23 (Fig 2C). Straight stigmata 2–5 per mesh (Fig. 2C).

Gut with a short oesophagus, a softly folded stomach (with an indeterminate number of folds), and an intestine that occupied almost 75% of left side of body. In some specimens, ovaries and testicular follicles covered the gut, but they were immature. We could not verify follicle cells and buoyancy of the oocytes.

Discussion

We detected the presence of *Ascidiella scabra* in the hull of R/V *ARA Puerto Deseado*. This is the first record of this species in the SW Atlantic, outside its native range. In a recent study updating exotic and cryptogenic species for this area, Schwindt et al. (2020) reported our finding but without the full data such as collection location of the specimens or taxonomic characters used in their identification. The reported distribution of the species typically encompasses the northeastern Atlantic, the North Sea, and the Mediterranean Sea (Berrill 1950; Millar 1970; Nishikawa and Otani 2004; Rocha et al. 2012; Nishikawa et al. 2014). During the 19th century, the species was reported outside of its native range in Nagasaki, Japan (Hartmeyer 1906), yet, this population has now completely disappeared (Nishikawa and Otani 2004). Thus, its presence in the hull-fouling community of R/V *ARA Puerto Deseado*, a vessel whose routes are confined the northern Antarctic Peninsula and southern Brazil in the SW Atlantic and the Southern Ocean (Meloni et al. 2020), constitutes an unexpected result and strongly suggests this species' presence in the region. Unless a hull-to-hull propagule transfer from a colonized overseas vessel had occurred, *A. scabra* must have been picked up by R/V *ARA Puerto Deseado* from a port or coastal area. Given that water temperatures in Antarctic coasts and islands visited by the vessel are presumably too low for this species, it is probable that propagules were picked up from a location on the Argentine coast. These results also suggest the potential of hull fouling on this and similar vessels to transport and possibly disperse *A. scabra* propagules regionally.

The fact that *A. scabra* has never been found previously in the SW Atlantic might be because it was mistaken for a similar non-indigenous ascidian, *A. aspersa*, which is common in this area (Tatián et al. 2010; Schwindt et al. 2020). The external appearances of these two species are indistinguishable. As already mentioned, it is necessary to dissect the specimens and count the total number of tentacles and longitudinal vessels, reliable traits to distinguish between the two species (Nishikawa et al. 2014). In the *A. scabra* specimens that we studied, there were in general fewer longitudinal vessels than oral tentacles. Only two of these specimens showed an equal number of longitudinal vessels and oral tentacles, but only on one side of the branchial sac (one specimen on the right and the other on the left side). In these specimens, however, the other half of the branchial sac presented fewer longitudinal vessels than oral tentacles. According to Nishikawa et al. (2014), both species have the potential to become invasive, although only *A. aspersa* has been reported so far in the ports of the SW Atlantic Ocean. Due to the similar external morphology of these two species and the possibility of finding them in sympatry, previous records of *Ascidiella* in this area should be reassessed. A detailed analysis, including inner features, is particularly recommended on specimens up to 6 cm in length, which is the overlap of the size ranges of these species.

Ascidiella scabra and *A. aspersa* share the same range in the northeastern Atlantic, North Sea, and Mediterranean Sea (Nishikawa and Otani 2004). *Ascidiella aspersa* has been established for more than 50 years in the SW Atlantic and is considered to be a pioneer species in the colonization of hard substrates in port areas (Tatián et al. 2010). Despite the frequent presence of *A. aspersa* in the SW Atlantic and the fact that R/V *ARA Puerto Deseado* spends a large part of the year in port at Mar del

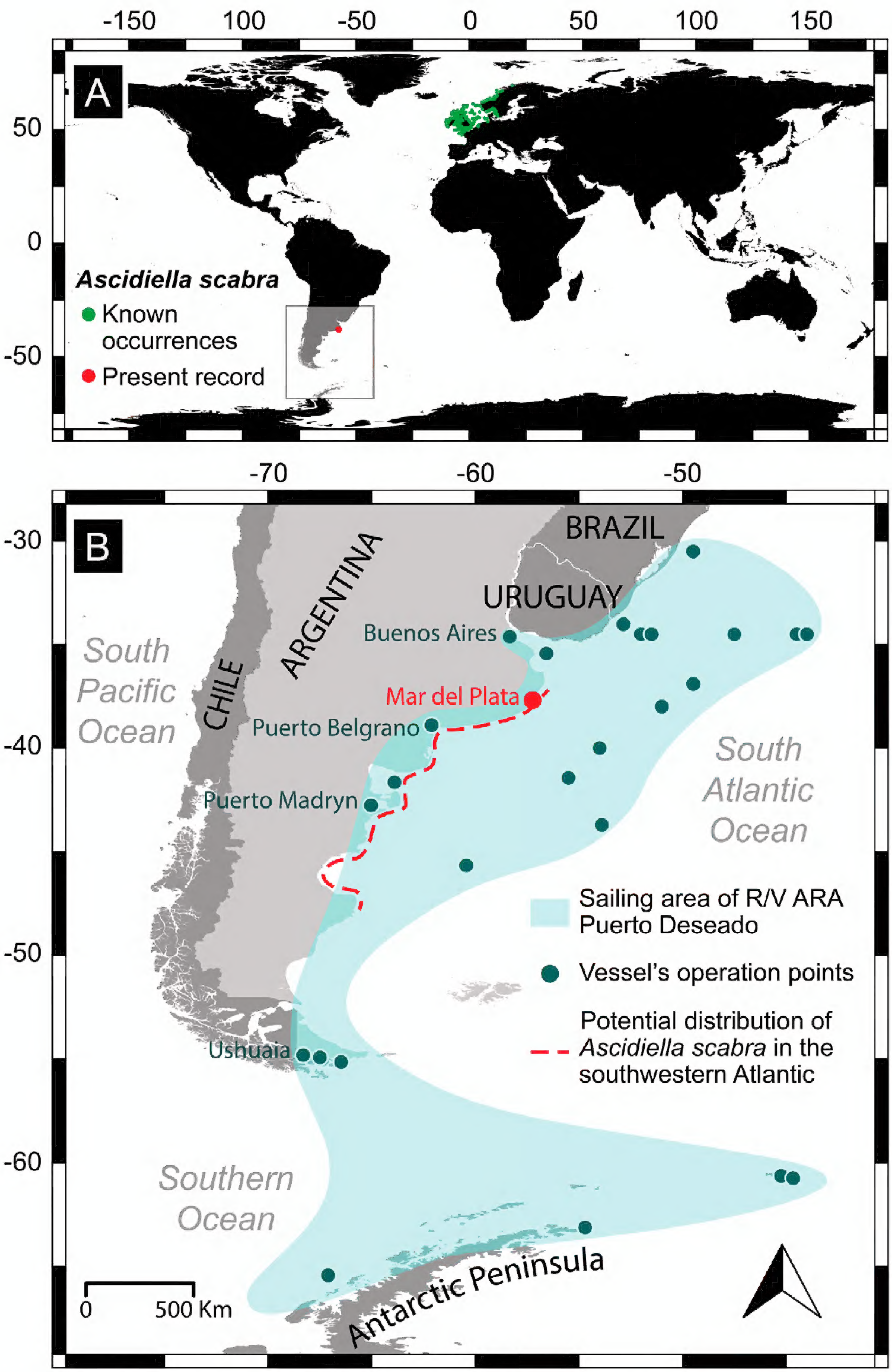

Figure 1. Distribution of *Ascidiella scabra* (Müller, 1776). **A.** Global distribution. **B.** Sampling port where *A. scabra* individuals were found attached to the hull of R/V *ARA Puerto Deseado* during in-water and dry-dock hull inspections in 2011. The approximate sailing area of the vessel during the last two years prior to sampling, based on specific operation points reported by the vessel in the same period (see Meloni et al. 2020 for details), and potential distribution of *A. scabra* are shown in the lower map. See main text for explanation of the potential distribution of *A. scabra* in the southwestern Atlantic and data sources for the species' global distribution.

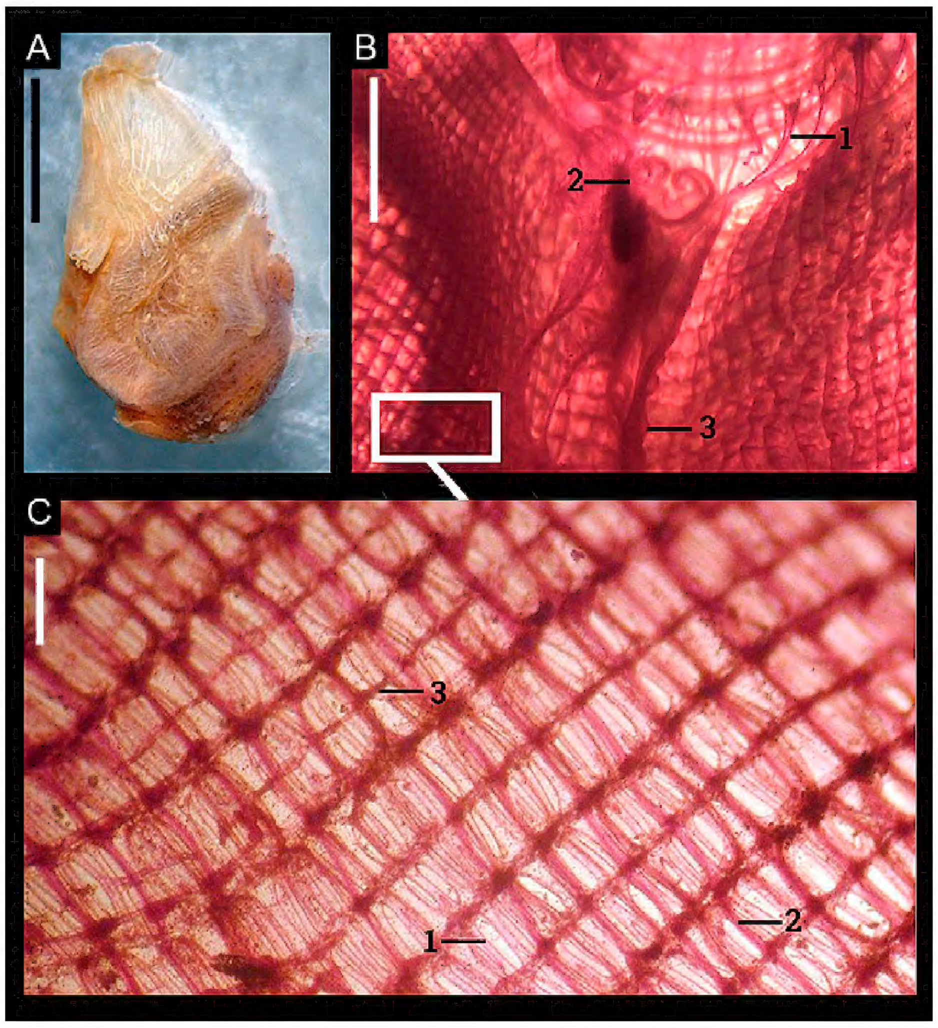

Figure 2. *Ascidiella scabra* (Müller, 1776). **A.** Specimen (MZUCVI 00003) without tunic from sample PD003-4 from bow-thruster gratins. **B.** Dissected specimen (MZUCVI 00007) from sample PD003-21 from sea-chest gratings (1 = oral tentacles, 2 = dorsal tubercle, 3 = dorsal lamina). **C.** Branchial sac detail of the same specimen (1 =stigmata, 2 = longitudinal vessel, 3 = transversal vessel). Scale bars: A = 2 cm; B = 0.5 cm; C = 0.2 cm.

Plata, we did not find specimens of *A. aspersa* but only *A. scabra* on the hull of this vessel. *Ascidiella scabra* and *A. aspersa* have similar environmental tolerances, including a preference for low salinity and the same temperature range for spawning. While *A. scabra* requires mean salinity values above 24 psu, with adults tolerating values as low as 15 psu, *A. aspersa* was found to withstand salinity values down to 18 psu (Dybern 1969a, 1969b). A spawning temperature range of 8–22 °C has been determined for both species (Dybern 1969a). Thus, it is likely that the potential distribution of *A. scabra* overlaps with that of *A. aspersa*, which is up to now known to extend from Mar del Plata to Puerto Deseado in the SW Atlantic, an area with a mean annual salinity of 35.7–32.7 psu and a mean annual temperature range of 9–16 °C (Tatián et al. 2010; Schwindt et al. 2014) (Fig. 1B).

Large commercial transoceanic vessels are well-known vectors for the primary introduction of marine species into coastal habitats (Sylvester et al. 2011; Reem et al. 2013), while smaller recreational, fishing, and other types of crafts may aid subsequent, secondary spread (Zabin et al. 2014; Leclerc et al. 2020). According to official sources, the port of Mar del Plata is largely dominated by domestic traffic consisting of fishing and oil-tanker vessels (ca. 610,000 t/y transported), with very small proportions of container (<5000 TEUs per year) and overseas vessels (<3% of total cargo moments) (Government of the Argentine Republic 2021). Our results indicate that domestic and regional traffic has a clear potential for the spread of *A. scabra* across coastal areas in the SW Atlantic. Colonization success and spread rate have been proposed to depend on the intensity of transport (generally increasing colonization success), biotic, and abiotic factors (with a variable effect on colonization success) in recipient communities (Blackburn et al. 2011). The southwestern Atlantic has a relatively low level of traffic between ports. Ports differ according to local (river mouths) and regional conditions. Among the latter, the temperature fluctuates in a latitudinal gradient of more than 18 degrees between Mar del Plata, in Buenos Aires Province, and Ushuaia, in Tierra del Fuego (Tatián et al. 2010). The present findings lead us to expect further *A. scabra* detections—whether from future or previously conducted samplings—in the SW Atlantic.

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Authors' Contributions

MM, NC, and FS obtained and processed the samples. DG carried out morphological analysis of samples and led the writing of the manuscript with support from MT and FS. AT contributed to the discussion of data. MT had the original idea of the study and supervised taxonomic observations. All authors contributed to the final version of the manuscript.

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